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(57) Abstract

Biocompatible adhesive conjugates useful in binding surfaces in the presence of water, methods of forming and using those adhesives are disclosed. The conjugates comprise a first proteinaceous substance having the ability to exhibit adhesive properties and a substantially non-antigenic polymeric substance. Uses of the novel conjugates including methods of affixing viable cells to substrates and methods of joining living tissue.

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CONJUGATED BIOADHESIVES

The present invention is directed to methods of conjugating bioadhesive materials such as peptides with non-antigenic polymers, as well as the resulting adhesive conjugates and their uses.

Background of the Invention

Bioadhesives are useful in laboratory and surgical procedures, for example, procedures involving skin grafts and the closing of skin incisions. As used herein, the term "bioadhesive" means an adhesive that is compatible with the metabolism, growth or function of living tissues, cells, and/or other biologically active moieties in vitro or in vivo. In addition to strong adherence, an important property of a bioadhesive is its viscosity. During a medical procedure, it is necessary that the adhesive have enough viscosity to remain on the desired site for a time sufficient to permit the surgeon to complete the procedure. Bioadhesives with low viscosity can tend to run and make surgical procedures very difficult.

Another important property is the rate at which the bioadhesive develops tackiness. This parameter is particularly important in procedures requiring the stabilization of skin grafts. If a bioadhesive develops tackiness immediately upon application, it can be very difficult for a surgeon to position and adjust a skin graft onto the desired site. An adhesive which develops tackiness at a slower rate can be clinically advantageous since it provides a surgeon with more time to position the

graft properly.

Examples of bioadhesives useful as cell and tissue adhesives are polyphenolic proteins isolated from the marine mussel Mytilus edulis. Methods of purifying this protein and its uses have been published in U.S. Patents 4,496,397, 4,585,585, 4,687,740, 4,808,702, and 5,108,923 which are hereby incorporated by reference. Analogs of the marine mussel extract have also been formed recombinantly as disclosed in U.S. patents 5,149,657 and 5,049,504 which are also hereby incorporated by reference. While these extracts and analogs are useful in immobilizing a variety of cell types, tissue slices, microorganisms and subcellular components, their non-human origin suggests that they may exhibit antigenicity levels that are undesirable for certain applications.

Another clinically important property of such bioadhesives is the time that it takes the initial preparation to become tacky and the duration of the time period in which they stay tacky. If the bioadhesives get tacky too quickly, they can be difficult to work with in certain clinical applications, e.g. during the placement of skin grafts.

Furthermore, such adhesive proteins have also been shown to cause tissue adhesions when such adhesions are not desired such as adhesions between abdominal walls and the cecum.

It would, therefore, be desirable to provide an improved bioadhesive having reduced antigenicity, improved

viscosity and a longer "tack time" to facilitate placement during surgical procedures. It would be particularly desirable to provide an improved bioadhesive which exhibits these properties without sacrificing adhesiveness.

Summary of the Invention

Embodiments of the present invention are directed to improved biocompatable adhesives useful in binding surfaces in the presence of water, as well as methods of forming and using these bioadhesives.

One embodiment of the present invention comprises a conjugate of a first proteinaceous substance having the ability to exhibit adhesive properties and a substantially non-antigenic polymeric substance. Other aspects of the present invention include methods of forming the conjugates, which preferably comprise a polyphenolic protein such as those described above.

According to one embodiment of the present invention, polyphenolic protein preparations are reacted with molar excesses of a suitable substantially non-antigenic polymeric substance, such as an activated polyalkylene oxide, under conditions sufficient to effect conjugation. Such conditions include reacting the substituents at temperatures of up to about 27°C (and in pharmaceutically acceptable buffer systems). As used herein, the term "molar excess" is meant to indicate the ratio of the number of moles of polymeric substance to the number of moles of the adhesive protein. This method has been found to provide a bioadhesive conjugate which is less

susceptible to antibody inhibitors and has a longer set-up time. Set-up time is the time required for the adhesive protein portion of the conjugate to polymerize or perform its permanent gluing function. The present invention advantageously provides methods for modifying adhesive proteins without subjecting the proteins to harsh conditions which could eliminate their activity.

Other aspects of the present invention comprise uses of the novel conjugates including methods of affixing viable cells to substrates and methods of joining living tissue.

Detailed Description

The present invention is directed to conjugates of a proteinaceous substance having the ability to exhibit adhesiveness and a substantially non-antigenic polymeric substance, methods of forming these conjugates, and their uses.

The starting material can be any of the starting materials in the above-referenced patents including commercially available purified forms of proteins obtained from the marine mussel Mytilus edulis, recombinantly formed analogs thereof, or other proteins, polypeptides or peptide sequences having lysine and tyrosine residues capable of exhibiting adhesive properties.

The substantially non-antigenic polymer substances included in the conjugates are preferably poly(alkylene oxides). Within this group of substances are

alpha-substituted polyalkylene oxide derivatives such as methoxypolyethylene glycols or other suitable alkyl-substituted derivatives such as C_1-C_4 alkyl groups. It is preferred, however, that the non-antigenic material be a monomethyl-substituted PEG homopolymer. Alternative polymers such as other polyethylene glycol homopolymers, polypropylene glycol homopolymers, other alkyl-polyethylene oxides, bis-polyethylene oxides and co-polymers or block co-polymers of poly(alkylene oxides) are also useful. In those aspects of the invention where PEG-based polymers are used, it is preferred that they have molecular weights of from about 200 to about 12,000. Molecular weights of about 2,000 to 7,000 are preferred and molecular weights of about 5,000 are particularly preferred.

Other substantially non-antigenic compounds such as polyvinyl pyrrolidone, dextran, complex carbohydrates, starches and other substantially non-antigenic compounds may also be conjugated with the protein using a covalent or non-covalent linkage.

Covalent modification of the protein material is preferred to provide a hydrolysis-resistant conjugate. The covalent modification reaction includes reacting a proteinaceous substance having the desired adhesiveness with a substantially non-antigenic polymeric substance under conditions sufficient to effect conjugations while maintaining the adhesive properties of the protein.

The polymers may be activated in order to effect the desired linkage with the protein substance. By

activation, it is understood by those of ordinary skill in the art that the polymer is functionalized to include a desired reactive group. Examples of such activation are disclosed in U.S. patents 4,179,337 and 5,122,614, which are hereby incorporated by reference. In the disclosures of these patents, the hydroxyl end groups of polyalkylene glycols are converted into reactive functional groups and thus activated.

According to one preferred embodiment, a polyphenolic protein fraction is modified with SC-PEG such as disclosed in the '614 patent, supra. This particularly preferred activated form of PEG for use in the present invention is poly(ethylene glycol)-N-succinimide carbonate. This activated polymer forms stable, hydrolysis-resistant carbamate (urethane) linkages with amino groups of the protein. Isocyanate-activated PEG's are also of use. While the references incorporated herein describe epsilon amino group modifications of lysine, other conjugation methods are also contemplated. Other amino acid modifications are also within the scope of the present invention. Covalent linkage by any atom between the protein or peptide and polymer is possible. Moreover, non-covalent conjugation such as lipophilic or hydrophilic interactions are also contemplated.

In order to prepare the adhesive protein fraction for the polymeric modification, the pH is preferably adjusted to about 6 - 9, and most preferably to about 7 - 8. In the case of polyphenolic proteins, the pH may be adjusted through a vigorous buffer exchange by dialyzing the protein against an appropriate salt buffer system. For

example, the buffer exchange can be conducted by placing the protein fraction in a dialysis bag suspended in a salt buffer and changing the buffer with fresh solution several times. According to one embodiment of the present invention, a polyphenolic proteinaceous substance is placed in a salt buffer having a pH of about 6-9, preferably 7-8, at temperatures which do not exceed 27°C, preferably in the range of from about 2 - 10°C. The salt buffer may, for example, comprise 100mM sodium phosphate at a pH of 7.5. Those skilled in the art will appreciate that if a lower pH is to be utilized, another salt, such as a phosphate, may be utilized.

A substantially non-antigenic polymeric substance, such as a poly(akylene oxide), is then reacted with the proteinaceous adhesive. The non-antigenic polymer can either be added as a solid or dissolved in a suitable buffer. For example, if the adhesive protein is dissolved in a borate buffer, an SC-PEG could also be dissolved in a borate buffer for more rapid mixing. Alternatively, the non-antigenic polymer can be added to the protein-salt solution as a solid. In the reaction mixture, the protein is reacted with an appropriate amount of the activated polymer, which is typically present in a molar excess. The polymeric excess will range from about 5 to about 125 fold molar excess and preferably from about 15 to about 50 fold molar excess of the polymer to the adhesive protein. The reaction is carried out at temperatures of from about 2 to 27°C, and preferably at temperatures of from about 2 - 10°C, over time periods ranging from a few minutes to as long as 12 hours, preferably for about 30 minutes to two hours, with occasional stirring. The resulting modified

adhesive protein has an average of about 1 - 25 strands of substantially non-antigenic polymer per molecule of adhesive protein, preferably about 8 - 12 strands, and most preferably about 8 - 10 strands. Depending upon the reaction conditions, the artisan can tailor the profile of the resultant conjugate. For example, large molar excesses of polymer reacted with the protein result in conjugates having relatively longer set-up times and somewhat less antigenicity than unmodified or slightly modified proteins. The inverse of the foregoing is also true. Smaller molar excesses reacted with the protein provide conjugates with shorter set-up times but higher antigenicity. It is desirable that about 1 to 25 strands, most preferably an average of about 8-10 strands of the non-antigenic polymer are conjugated with each mole of protein.

One advantage of the modification process disclosed herein is that it can be carried out at relatively mild reaction conditions which will not adversely affect the bioadhesive protein. The reaction is then stopped by adding a molar excess of a compound which reacts quickly with the polymer such as a molar excess of glycine.

Following the conjugation reaction, the desired product is recovered using known techniques and purified using column chromatography or similar apparatus if necessary. For example, excess reagents can then be removed from the reaction mixture by the same dialysis procedure described above. The modified adhesive protein may also be lyophilized to facilitate storage and handling.

In a typical application of the present invention, a purified form of a recombinantly formed polyphenolic protein, having a molecular weight of about 25 - 120 KD, is conjugated with a substantially non-antigenic polymer in the manner discussed herein. The conjugate is then treated with a non-polymerizing concentration of tyrosinase from Streptomyces antibioticus under conditions sufficient to modify at least some of the tyrosine residues on the protein to dihydroxyphenylalanine (DOPA). For example, a base protein can be dissolved in acetic acid solution and dialyzed against a buffer, for example, a 0.1M sodium phosphate buffer, pH 7.0 on ice. This adhesive protein solution can then be reacted with substantially pure tyrosinase in the presence of a reductant, such as ascorbic acid. At this step in the process, it is important to include a reductant in order to prevent the DOPA from forming quinones which could crosslink and set. It has been found that by including a suitable reductant, that about 50% of the tyrosine sites on the base protein can be converted to DOPA. The hydroxylated protein is then preferably filtered and lyophilized so that it is suitable for storage for later use. This results in a stable "pre-adhesive" DOPA protein which can be lyophilized and stored for extended periods.

At the time of use, the "pre-adhesive" DOPA form of the adhesive protein conjugate is enzymatically activated by oxidation of at least some of the DOPA to a reactive quinone, preferably by reaction with the same tyrosinase enzyme but without the reductant. The quinones crosslink to yield the cured adhesive protein.

The adhesive protein conjugates of the present invention are preferably presented to the user as a two-component system comprising the PEG-DOPA protein and the enzyme catalyst requiring the end user to mix the components prior to use. As used herein, the term "adhesive protein" includes the starting material, conjugated and unconjugated forms, as well as forms of the protein which have or have not been hydroxylated and/or subjected to oxidation prior to use. Thus, as used herein, the term "adhesive protein" includes starting materials which are inherently capable of exhibiting, but may not yet exhibit, adhesive properties.

Another embodiment of the present invention comprises a method of affixing viable cells to a substrate comprising the step of applying a bioadhesive conjugate prepared according the present invention to a substrate prior to contacting the viable cells to the substrate. The bioadhesive conjugates of the present invention are useful in affixing cells for a tissue culture or non-tissue culture to substrates, including but not limited to, plastic, glass, metals, microporous filters, and synthetic or alloplastic materials that may be used in tissue or prosthetic graft procedures.

A still further embodiment of the present invention comprises a method of joining a first group of living cells to a second group of living cells comprising the steps of (1) applying an adhesive proteinaceous conjugate comprising a polyphenolic protein and a substantially non-antigenic polymer to said first group of living cells; and (2) contacting said first group of living

cells with said second group of living cells.

EXAMPLE 1

MODIFICATION OF ADHESIVE PROTEIN WITH SC-PEG

In this example, PEG-bioadhesive conjugates were prepared using 3 different molar excesses of SC-PEG. PEG-ADP10 conjugates were prepared with a 10-fold molar excess of PEG while PEG-ADP20 and PEG-ADP50 conjugates were made using a 20-fold and a 50-fold molar excess, respectively. The bioadhesive used in this example was prepared in accordance with the method disclosed in Biotechnology Progress Vol.6, 171-177 (1990) which is hereby incorporated by reference and encoded by the plasmid YpGX477.

Five milligrams of the adhesive, along with 1 ml borate buffer pH 8.0, was added to each of 3 vials. Thereafter, 3.85 mg of PEG (10x molar excess) was added to the first vial, 9.6 mg of PEG (20x molar excess) was added to the second vial and 24.06 mg (50x molar excess) was added to the last vial. The reaction mixtures were stirred for 1 hour on ice, i.e., at about 4°C. A Centricon 30 was used to remove the excess PEG. The modification was checked by polyacrylamide gel electrophoresis using a buffer that contains sodium dodecyl lauryl sulfate (SDS-PAGE). While the unmodified adhesive protein had a molecular weight of 75,000, the conjugates formed with PEG-ADP10 had a molecular weight of 100,000, the PEG-ADP20 conjugate had a molecular weight of 150,000, and the PEG-ADP50 conjugate had a molecular weight of >200,000. The PEG-ADP10, PEG-ADP20 and PEG-ADP50 conjugates were determined to be about 8 strands, about 16 strands and

about 40 strands, respectively, by TNBS assay.

EXAMPLE 2

HYDROXYLATION OF ADHESIVE CONJUGATE

About 500 mg of PEG-ADP10, prepared in the same manner as in Example 1, was carefully added to 0.1% acetic acid to give a concentration of about 5mg/ml in a 200ml Erlenmeyer flask. This solution was mixed at room temperature until the protein was completely solubilized.

The protein was then dialyzed twice against 4 liters of 0.1M sodium phosphate buffer, pH 7.0 at 4°C, preferably for a minimum of 4 hours each.

The protein-buffer solution, which contains about 4-5mg/ml of protein, was then placed in a 2-liter plastic Erlenmeyer flask. Ascorbic acid, preferably mixed fresh with 0.88 grams of acid in 5ml of water, was added in an amount equal to 1/40th the volume of the protein solution so that the final concentration of ascorbic acid was 0.25mM. Then tyrosinase was added to give a final concentration of enzyme of 1 U/ml enzyme activity. One activity unit (U) is defined as the amount of enzyme which catalyzes the oxidation of 1μ mole dopa/minute, see Gene Vol. 37, pp 101-110 (1985). The solution wad incubated at 28°C for about 90 minutes during which time it was shaken, 1 hour at 200 rpm and 30 minutes at 55 rpm. The reaction was then terminated by the addition of 2.5M HCl in an amount equal to about 1/20th of the total volume.

The samples were then dialyzed against 0.01% acetic acid at 4°C, analyzed using a nitrite assay, concentrated, filtered through a 0.22μ Corning disposable filter (cellulose acetate membrane), lyophilized and stored at -20°C.

EXAMPLES 3 - 5

The performance of the PEG-ADP10 conjugated bioadhesive, having about 8 PEG strands per molecule and prepared in accordance with Example 1 , was compared to a non-conjugated form of the same bioadhesive in the following three animal models:

Skin Graft Adherence
Linear Dorsal Skin Incisions
Peritoneal Adhesions

For each animal model, the adhesive was prepared as follows:

USE	ADHESIVE CONCENTRATION		AMOUNT APPLIED	
	PROTEIN (mg/ml)	ENZYME (units/ml)	VOLUME (ml)	PROTEIN (mg)
SKIN GRAFT	20	4	0.5	10
SKIN INCISION	40	8	0.01	0.4
PERITONEAL ADHESION	28	11.2	0.2	5.6

Skin Graft Adherence

Two rabbits were utilized. Each rabbit had two grafts removed and eight strips of autologous skin replaced. Mussel adhesive protein (MAP) used in Example 1 was used on half the grafts, and conjugated bioadhesive of the present invention was used on the other half.

Partial-thickness (0.015") skin grafts (4" x 7") were removed from each dorsolateral aspect of a surgically-prepared rabbit. The grafts were cut into 1" x 3" strips and reapplied to the wound bed as autografts after hemostasis was achieved. The biological adhesives were used on the wound bed to enhance the adherence of the graft to the wound. In order to control the application of adhesive, a plastic template was laid over the wound with a 1.25" x 3/5" window removed at the site designated to receive the adhesive.

Adhesive was sprayed onto the wound bed as a premixed mixture using a template. The graft strip was placed on the wound after a 30-second delay. After all graft strips had been placed, each wound was dressed with petroleum jelly gauze followed by dry gauze, a circumferential wrap of Kling® gauze, and finally, an Ace® bandage.

Graft adherence was measured after 5.5 hours as the graft was peeled off the wound (180°) at the constant rate of 8.8 cm/min, using a calibrated strain gauge. The mean peel force (g) was determined for each sample to be 9.9 ± 1.6 gm for the MAP and 8.0 ± 2.2 gm for the

conjugated form of the present invention. Thus, the addition of PEG to the adhesive protein does not interfere with the adhesive characteristics of the protein.

Linear Dorsal Skin Incisions

Ten surgically-prepared rats were utilized. Two paravertebral dorsal incisions were made on each of the rats. The incisions were 2 cm in length, were separated by 3 cm, and involved the full-thickness of skin including the underlying panniculus carnosus muscle. Application of the pre-mixed adhesives was accomplished with the use of a syringe and 25-gauge needle. After application of each adhesive, the wound edges were approximated (held together by hand) for two minutes. The animals were kept sedated until the scheduled time of analysis at 2 hours.

To analyze the strength of the closed incisions, a fixed clamp was attached to the animal's skin on one side of the test incision through an additional small incision created at the time of surgery. The skin on the opposite side of the test incision was attached in a similar fashion to a movable clamp which was distracted from the fixed clamp at a rate of 8.8 cm/minute. The force (g) required to disrupt the incision was monitored with a strain gauge and plotted on a strip chart recorder. The highest force endured by the incision prior to disruption was recorded as the incisional strength. The average force for the standard MAP was 48 ± 18 gms while the average force for the conjugate of the present invention was 50 ± 21 gms. Thus, the addition of PEG to the adhesive protein does not interfere with the adhesive characteristics of the protein.

Peritoneal Adhesions

Twenty-four rats were utilized. The rats were anesthetized and prepared for abdominal surgery. The skin was incised along the midline and reflected from the abdominal wall. The abdomen was also incised along the midline. The right abdominal wall was reflected, and a 1 x 2 cm rectangle marked on its peritoneal surface 1 cm lateral to the incision. The peritoneum at the site of the marking was cut with a scalpel blade and the peritoneum removed from the abdominal wall within the rectangle.

The rat's cecum was elevated, and a 1 x 2 cm area on the anterior surface of the distal end was abraded by rubbing with dry gauze until standardized petechial hemorrhaging occurred. The peritoneal and cecal defects were each exposed to air for 15 minutes before the adhesive seal was applied. 0.2 mls of adhesive was applied by syringe to cover each defect. Half of the animals received standard MAP, and half were treated with the conjugate of the present invention. The adhesive seal was allowed to set for 5 minutes before the abdominal wall was returned to its original position and the two sealed wounded placed in contact with each other. The abdominal wall was closed with running polypropylene suture, and the skin closed as a separate layer in the same manner. Each animal was bandaged and wrapped circumferentially with tape. When the abdomens were closed, the wound surfaces with their adhesive seals came in contact with each other. Without an application of adhesive, 100% of the animals would develop an adhesion between the two wound surfaces.

Seven days later, the animals were euthanized and their abdomens explored for the presence of an adhesion between the abdominal wall defect and the injured cecum. Ten of the twelve rats treated with standard MAP developed peritoneal adhesions while none of the rats treated with the conjugate of the present invention developed these adhesions.

WE CLAIM:

1. A biocompatable adhesive comprising a conjugate of a first substance having adhesive properties and a substantially non-antigenic polymeric substance.

2. An adhesive according to claim 1 wherein said first substance comprises a polyphenolic protein .

3. An adhesive according to claim 1 wherein said substantially non-antigenic polymeric substance is a poly(alkylene oxide).

4. An adhesive according to claim 3 wherein said poly(alkylene oxide) is an alpha-substituted polyalkylene oxide derivative.

5. An adhesive according to claim 3 wherein said poly(alkylene oxide) is selected from the group consisting of polyethylene glycol homopolymers, polypropylene glycol homopolymers, alkyl-capped polyethylene oxides, bis-polyethylene oxides and copolymers or block copolymers of poly(alkylene oxides).

6. An adhesive according to claim 5 wherein said polymer has a molecular weight of from about 200 to about 12,000.

7. An adhesive according to claim 6 wherein said polymer has a molecular weight of from about 2,000 to about 7,500.

8. An adhesive according to claim 1 wherein said polymer is linked to said first substance with a carbamate (urethane) linkage.

9. An adhesive according to claim 1 wherein said polymer is linked to said first substance with a covalent linkage.

10. An adhesive according to claim 2 wherein said polyphenolic protein is of recombinant origin.

11. An adhesive according to claim 1 wherein said first substance comprises an extract from a marine animal.

12. An adhesive according to claim 11 wherein said first substance comprises an extract from the marine mussel genus Mytilus.

13. An adhesive according to claim 1 wherein aid substantially non-antigenic polymeric substance is selected from the group consisting of polyvinyl pyrrolidone, complex carbohydrates, dextran, starch, or mixtures thereof.

14. A method of preparing a biocompatible adhesive comprising reacting a first substance comprising an adhesive protein with a substantially non-antigenic polymeric substance under conditions sufficient to effect conjugation of said first substance and said polymeric substance.

15. A method according to claim 14 wherein said polymer is a poly(alkylene oxide).

16. A method according to claim 15 wherein said polyalkylene oxide is an alpha-substituted polyalkylene oxide derivative.

17. A method according to claim 16 wherein said poly(alkylene oxide) is a polyethylene glycol.

18. A method according to Claim 14 wherein said reacting step comprises providing a molar excess of said substantially non-antigenic polymeric substance relative to said first substance.

19. A method according to Claim 18 wherein said reacting step comprises providing about a 15 - 50 fold molar excess of said substantially non-antigenic polymeric substance relative to said first substance.

20. A method according to claim 14 wherein said reacting step is conducted at temperatures of up to about 27°C.

21. A method according to claim 20 wherein said reacting step is conducted at temperatures of about 2 - 10°C.

22. A method according to claim 13 wherein said first substance comprises a polyphenolic protein.

23. A method of affixing viable cells to a substrate comprising the step of applying a bioadhesive conjugate comprising a first substance comprising an adhesive protein and a substantially non-antigenic polymeric substance to

said substrate.

24. A method according to claim 23 wherein said first substance comprises a polyphenolic protein.

25. A method according to claim 15 wherein said substrate non-antigenic polymer substrate is a poly(alkylene oxide).

26. A method according to claim 16 wherein said poly(alkylene oxide) is selected from the group consisting of polyethylene glycol homopolymers, polypropylene glycol homopolymers, alkyl-capped polyethylene oxides, bis-polyethylene oxides and copolymer or block copolymers of poly(alkylene oxides).

27. A method of joining a first group of living cells to a second group of living cells comprising the steps of:

applying an adhesive proteinaceous conjugate comprising a polyphenolic protein and a substantially non-antigenic polymer to said first group of living cells; and

contacting said first group of living cells with said second group of living cells.

28. A method according to claim 27 wherein said substantially non-antigenic polymer substrate is a poly(alkylene oxide).

29. A method according to claim 28 wherein said poly(alkylene oxide) is selected from the group consisting of polyethylene glycol homopolymers, polypropylene glycol homopolymers, alkyl-capped polyethylene oxides, bis-

polyethylene oxides and copolymer or block copolymers of poly(alkylene oxides).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/06782

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :A61K 47/48; C07K 17/08; CO8H 1/00
US CL :530/410, 812; 525/54.1, 55; 527/200; 424/78.08, 78.31; 514/2,12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/410, 812; 525/54.1, 55; 527/200; 424/78.08, 78.31; 514/2,12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,179,337 (DAVIS ET AL) 18 December 1979, entire document.	1-29
Y	US, A, 5,122,614 (ZALIPSKY) 16 June 1992, see entire document.	1-29
Y	Trends in Biotechnology, Volume 8, No. 2, issued February 1990, R.L. Strausberg et al., "Protein-Based Medical Adhesives", pages 53-57, see entire document.	1-29
Y	US, A, 4,808,702 (WAITE) 28 February 1989, see entire document.	1-29
Y	US, A, 5,049,504 (MAUGH) 17 September 1991, see entire document.	1-29

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
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Date of the actual completion of the international search
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Date of mailing of the international search report
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,015,677 (BENEDICT ET AL) 14 May 1991, see entire document.	1-29

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Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, LIFESCI, EMBASE, MEDLINE, CAS, BIOTECHDS, WPIDS
search terms: polyalkylene or polyethylene or polypropylene or polyvinyl or methoxypolyethylene, protein# or enzyme# or polypeptide# or bioadhesive# or polyphenolic, link? or crosslink? or conjugat?